cultured with DAPT exhibited a less pronounced fall of mRNA and protein level of type II collagen and aggrecan, and a minor increase of type I collagen mRNA. In order to further confirm the participation of Notch1 in the dedifferentiating process of iMACs, we transiently transfected the cultured cells with an expression plasmid encoding a constitutively active form of Notch 1 receptor (DE form) which is constitutively cleaved by the γ-secretase, releasing the transcriptionally active nuclear form of Notch, ICv. After 24 hours of transfection we observed a strong diminution of type II collagen amount, detected by western blot, whereas the Notch1 full length construction as well as the empty vector had no detectable effect. This diminution was abolished in presence of the γ-secretase inhibitor DAPT (2,3μM).
Conclusions: These data show that the Notch pathway is critical in the dedifferentiation process of articular chondrocytes. We suggest that modulating the Notch pathway could be a novel approach in the treatment of OA.

A13

TG2-CATALYZED TRANSAMIDATION OF K3 AND Q102 RESIDUES TRANSFORMS LATENT S100A11 CALGRANULIN INTO AN INDUCER OF MATRIX CATABOLISM AND HYPERTROPHIC DIFFERENTIATION IN CHONDROCYTES

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Purpose: Alterations in chondrocyte differentiation, including chondrocyte hypertrophy, promote dysregulated articular cartilage matrix repair and tissue failure in aging and osteoarthritis (OA). IL-8 and TNFα induce chondrocyte hypertrophy, dependent on both release of ligands of receptor for advanced glycation endproducts (RAGE) and expression of the protein crosslinking enzyme transglutaminase 2 (TG2). S100/calgranulins are prototypical RAGE ligands. S100A11 release, markedly upregulated in human OA chondrocytes in situ, is induced by IL-8 and TNFα in vitro. S100A11 is crosslinked by TG2-catalyzed transamidation to change conformation and multimerize, with potential for altered signaling through the calgranulin receptors RAGE and CD36. Hence, we tested the hypothesis that TG2 post-translationally modifies S100A11 released by chondrocytes, triggering articular chondrocyte maturation to hypertrophy.
Methods: We studied not only human articular chondrocytes but also RAGE knockout, TG2 knockout, and congenic wild type mouse femoral head articular cartilage explants. Adapting methods of Salvat et al (OA Cartilage 13:243, 2005), we also isolated immature murine articular chondrocytes confirmed to uniquely express type II collagen and aggrecan. We generated TG2 transamidation site point mutants (K3L, Q102N, and both) of human S100A11 and as a control a K52R S100A11 site mutant. We also made single and double mutants for human TG2 GTP binding (K173L) and transamidation catalytic (C277G) sites.
Results: RAGE knockout chondrocytes and femoral head cartilage explants retained the capacity for IL-1-induced NO and GAG release, ADAMts4 and 5 expression, and decreased proteoglycans (PG) synthesis. In contrast, we confirmed that IL-8 and TNFα required RAGE to induce type X collagen in chondrocytes (J. Immunol. 175:12, 2005), and TNFα-induced GAG release was blunted in RAGE knockout cartilage explants. Nanomolar S100A11 failed to induce type X collagen and other markers of chondrocyte hypertrophy in both RAGE and TG2 knockout mouse chondrocytes and in human chondrocytes in which TG2 was knocked down by RNAi. Conversely, nanomolar exogenous TG2 markedly accelerated chondrocyte hypertrophy in response to recombinant S100A11, an effect abrogated by the TG2 C277G but not K173L mutation. The K3R/Q102N S100A11 mutation but not the K52R S100A11 mutation removed the capacity of S100A11 to induce chondrocyte hypertrophy. Wild type but not K3R/Q102N mutant S100A11 stimulated a 69% increase in GAG release in wild type mouse femoral head cartilage explants ($P<0.05$). Wild type S100A11 also failed to induce GAG release in either RAGE or TG2 knockout explants. Last, wild type but not K3R/Q102N mutant S100A11 induced decreased PG synthesis in cultured chondrocytes. In contrast, wild type S100A11 induced 80-120% increase in PG synthesis in TG2 knockout chondrocytes compared to wild type ($P<0.05$).
Conclusions: TG2-catalyzed transamidation of K3 and Q102 residues transforms latent S100A11 calgranulin to a conformational state in which S100A11 induces hypertrophic differentiation, GAG release, and decreased PG synthesis. Conversely, S100A11 fails to do so in the absence of RAGE or TG2(or the capacity to be transamidated and crosslinked by TG2), conditions under which S100A11 actually is anabolic as evidenced by PG synthesis. Our results suggest a novel model by which chondrocyte hypertrophy and a matrix catabolic program in OA cartilage are switched on by pericellular TG2-induced conformational change in cytokine-inducible secreted calgranulins.

A14

INJECTED TIMP-3 PROTECTS CARTILAGE IN A RAT MENISCAL TEAR MODEL

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Purpose: The aim of this study was to determine whether intra-articularly delivered TIMP-3 protects cartilage in a rat meniscal tear model of osteoarthritis. TIMP-3, a protein of 24 kD that binds to extracellular matrix, is a potent inhibitor of the metalloproteases that degrade aggrecan and type II collagen. It also inhibits TACE, the major enzyme that generates the soluble form of TNF. The protein consists of two discrete domains, and the inhibitory function is carried out by the N-domain. The N-domain has been shown to block IL-1-driven glycosaminoglycan (GAG) release in cartilage explants.
Methods: Two forms of TIMP-3 were produced: His-tagged full-length was made in CHO cells, while the N-domain was made in E. coli and refolded. Both forms were tested for efficacy against metalloproteases in vitro, against TNF release in cell culture, and against GAG release in bovine nasal cartilage explants. They were then tested in a model of osteoarthritis in which symptoms are induced by cutting the meniscus in knees of male Lewis rats. The test proteins, rat serum albumin, or vehicle were injected intra-articularly every other day for 3 weeks, starting one day prior to the surgery. The volume injected was 60 ul; the amount of protein was 100 ug for full-length TIMP-3 and albumin, 62 ug for the N-domain. At the end of the 3-weeks, joints were fixed, embedded, sectioned and stained, and then analysis was carried out for GAG content, cartilage width at various sites, osteophytes and bone lesions.
Results: Full-length TIMP-3 inhibited MMP-13 by about 80% at a ratio of 1:1, while the N-domain did so only at a 20-fold higher concentration. However, the two forms were similarly effective against other metalloproteases tested, and in blocking TNF release from a mouse monocytic cell line (IC50 about 75 nM in the cell assay). Both forms blocked IL-1-induced GAG release from bovine nasal cartilage, with IC50s of about 100 nM and 30 nM for full-length and N-domain, respectively. In the rat meniscal tear model, both forms reduced symptoms, with the most dramatic effects seen in the rats that received the full-length protein: 94% reduction in significant tibial cartilage degeneration width, 53% reduction in medial tibial osteophyte score, 67% reduction in bone score, and 57% reduction in total joint score.

Conclusions: We found that N-domain and full-length TIMP-3 are similarly effective in most in vitro and cell-based assays, although full-length was more potent against MMP-13. The N-domain was more effective in blocking IL-1-induced GAG release from bovine nasal cartilage explants. Both forms, but most dramatically the full-length protein, inhibited cartilage degradation, osteophyte growth and bone lesions in a rat meniscal tear model of osteoarthritis. The greater efficacy seen with the full-length form could be due to its greater potency against MMP-13.

A15

ELEVATED EXTRACELLULAR MATRIX PRODUCTION AND GAG DEGRADATION UPON BMP-2 STIMULATION POINT TOWARDS A ROLE FOR BMP-2 IN CARTILAGE REMODELING

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Purpose: BMP-2 has often been proposed as a good tool for cartilage repair and as a strong stimulant of chondrogenesis. Cells modified to overexpress BMP-2 and scaffolds coated with BMP-2 have been placed into a cartilage defects for reparative purposes. However, it is unknown what BMP-2 does on intact cartilage that is present in the treated joints. To study the effect of BMP-2 on intact cartilage in vivo, we overexpressed BMP-2 in normal murine knee joints and evaluated the effects on proteoglycan synthesis and degradation.
Methods: C57Bl/6 mice were injected intra-articularly with an adenovirus overexpressing BMP-2. Mice were injected i.p. with $35SO_4^{2-}$ 24 hours before sacrifice. After 3, 7 and 21 days knee joints were isolated for histology to perform Safranin O/Fast Green staining and autoradiography to assess anabolic effects of BMP-2. Immunohistochemical staining of the aggrecan neo-epitopes VDIPEN and NITEGE was carried out to investigate GAG degradation. Histological quantification of staining in patellar and tibial cartilage was performed with a computerized imaging system. In addition, patellar and tibial cartilage were isolated for measurement of proteoglycan (PG) synthesis by means of $35SO_4^{2-}$ incorporation or RNA isolation for quantitative PCR.
Results: BMP-2 overexpression resulted in altered chondrocyte appearance, which appeared to be larger than chondrocytes in control cartilage. Furthermore, BMP-2 stimulated PG-synthesis in patellar cartilage significantly on all days (up to two fold) and in the tibia on day 21. Stimulation was less prominent in tibial cartilage than in patellar cartilage. On mRNA level collagen type II expression had increased on all days in the patella with the highest expression on day 7 (17-fold) Aggrecan expression showed the same pattern with a a 13-fold increase on day 7. On the tibia collagen type II expression had increased 12-fold on day 7 and 14-fold on day 21 and aggrecan expression was elevated 15-fold on day 7 and 12-fold on day 21.In addition to stimulation of extracellular matrix production, BMP-2 overexpression also resulted in MMP- as well as ADAMTS-mediated cartilage degradation. VDIPEN staining (indicating MMP activity) was elevated upon BMP2 stimulation on day 3 on tibial cartilage and on day 3 and 7 in patellar cartilage, but no longer by day 21. NITEGE staining (indicating aggrecanase activity) was not found on day 3, but this is likely due to elevated MMP-activity cleaving off the NITEGE epitope. On day 7 NITEGE staining had increased 2-fold in the lateral tibial condyle and 3,5 fold in patellar cartilage, which was still increased 2-fold on day 21. On RNA levels we found elevated MMP3 expression on both tibia and patella, and elevated ADAMTS4 expression in the patella. BMP-2 overexpression did not result in detectable cartilage damage in Safranin O/Fast Green stained sections.

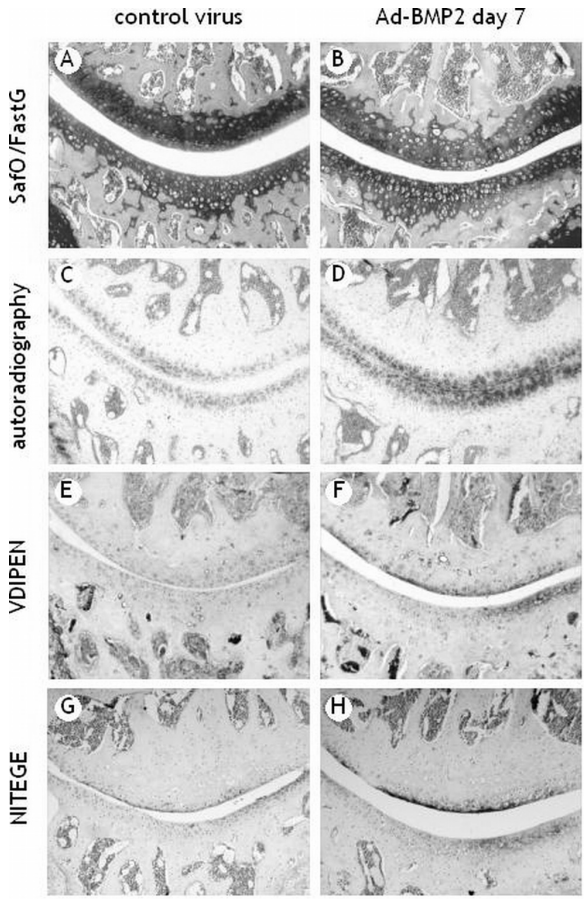

Fig. 1

Conclusions: Adenoviral overexpression of BMP-2 shows that BMP-2 is able to elevate proteoglycan synthesis in cartilage and stimulates collagen type II and aggrecan mRNA expression. At the same time increased catabolic activity was observed indicated by elevation of VDIPEN and NITEGE neo-epitopes. These data show that BMP2 not only boosts matrix synthesis in normal cartilage but that BMP-2 increases matrix turnover. Increased matrix turnover might be functional to replace matrix molecules in the repair of a damaged cartilage matrix.

A16

DIFFERENTIAL ROLES OF IKKα AND IKKβ IN CHONDROGENESIS AND OA INFLAMMATION REVEALED BY RETROVIRAL MEDIATED RNA INTERFERENCE

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Purpose: IKKα and IKKβ are essential kinases for activating NF-κB transcription factors that regulate cellular differentiation and inflammation. Each IKK was targeted for knockdown (KD)